2. interacts with a CDR; or

3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another; [and]

g. for any non-homologous import antibody amino acid residue which is [reasonably] expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and

h. preparing a humanized antibody variable domain having amino acid sequences determined in steps a-g.

2. (Amended) The method of claim 1, having an additional step of determining if any such non-homologous residues are exposed on the surface of the consensus human antibody variable domain or buried within it, and if the residue is exposed, retaining the consensus residue.

3. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the import antibody variable domain amino acid sequence for glycosylation sites, determining if any such glycosylation site is reasonably expected to affect the antigen binding or affinity of the antibody, and if so, substituting the glycosylation site into the consensus amino acid sequence.

4. (Amended) The method of claim 1, having the additional steps, which may be taken between any two steps in the method of claim 1, of searching the consensus variable domain amino acid sequence for glycosylation sites which are not present at the corresponding amino acid in the import antibody amino acid sequence, and if the glycosylation site is not present in the import sequence, substituting the import amino acid residues for the amino acid residues comprising the consensus glycosylation site.

5. (Amended) The method of claim 1, having an additional step which comprises aligning import antibody and consensus antibody FR amino acid sequences, identifying import antibody FR amino acid residues which are non-homologous with the aligned consensus FR sequence, and for each such non-homologous import antibody FR amino acid residue, determining if the corresponding consensus antibody amino acid residue represents a residue which is highly conserved across all species at that site, and if it is so conserved, preparing a humanized antibody which comprises the consensus antibody amino acid residue at that site.

7. (Amended) A method for making a humanized antibody comprising providing [at least a portion of] a non-human antibody variable domain amino acid sequence which is desired to be humanized (import antibody) having a CDR and a FR, obtaining the amino acid sequence of at least a portion of a consensus human antibody variable domain having a CDR and a FR, substituting the non-human CDR for the human CDR in the consensus human antibody variable domain, and then substituting an amino acid residue for the consensus amino acid residue at at least one of the following sites:
4L, 35L, 36L, 38L, 43L, 44L, 46L, 58L, 62L, 63L, 64L, 65L, 66L, 67L, 68L, 69L, 70L, 71L, 73L, 85L, 87L, 98L, 2H, 4H, 24H, 36H, 37H, 39H, 43H, 45H, 49H, 58H, 60H, 68H, 69H, 70H, 73H, 74H, 75H, 76H, 78H, 91H, 92H, 93H, and 103H.

[Please add the following new claims 17-21:]

--17. A method of using a consensus human antibody variable domain amino acid sequence in the preparation of a humanized antibody.-- 112/2nd no method steps.

--18. In a method for making a humanized antibody variable domain, the improvement consisting of using consensus human antibody variable domain amino acid sequence.--

--19. A method for making an improved antibody, comprising amino acid sequence from a non-human (import) antibody and a human antibody, comprising the steps of:

a. obtaining the amino acid sequences of at least a portion of an import antibody variable domain and of a consensus human antibody variable domain;

b. identifying Complementarity Determining Region (CDR) amino acid sequences in the import and the human amino variable domain sequences;

c. substituting an import CDR amino acid sequence for the corresponding human CDR amino acid sequence;

d. aligning the amino acid sequences of a Framework Region (FR) of the import antibody and the corresponding FR of the consensus antibody;

e. identifying import antibody FR residues in the aligned FR sequences that are non-homologous to the corresponding consensus antibody residues;

f. determining if the non-homologous import amino acid residue is reasonably expected to have at least one of the following effects:

1. non-covalently binds antigen directly,

2. interacts with a CDR; or

3. participates in the V_L - V_H interface by affecting the proximity or orientation of the V_L and V_H regions with respect to one another;

g. for any non-homologous import antibody amino acid residue which is reasonably expected to have at least one of these effects, substituting that residue for the corresponding amino acid residue in the consensus antibody FR sequence; and

h. preparing an improved, humanized antibody having amino acid sequences determined in steps a-g; and

i. evaluating the antigen binding or immunogenicity of the improved, humanized antibody with respect to the parental antibody.--

--20. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the manufacture of the antibody.--

--21. A method comprising, following the identification of an antibody by the method of any one of claims 1, 7, or 17-19, the expression of nucleic acid encoding the antibody.--

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Remarks

Claims 1-13, and 17-21 are presented herein for examination. Reconsideration of the outstanding rejections is respectfully requested for the reasons that follow. A request for a one-month extension of time to respond is submitted herewith, bringing the due date for this response to 5 February 1993. This response is timely filed.

Amendments

Claims 1, 3, 4, 5 and 7 have been amended to indicate that an import antibody is a non-human antibody which is desired to be humanized. Support for this language is found in the specification at page 6, line 27 to page 7, line 3.

Claim 1, step (f) has been amended to clarify that the word "participates" in the V_L - V_H interface means to affect the proximity or orientation of the V_L and V_H regions with respect to one another. Support for this amendment is found on page 15, lines 30-32. New step (h) has been added to claim 1, directed to the physical step of preparation of a humanized antibody variable domain. Support for this step appear throughout the specification.

Claims 3 and 4 have been amended to provide that the additional steps may be taken between